Pedobacter agri sp. nov., from soil

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A Gram-negative strain, PB92^T, which belongs to the family *Sphingobacteriaceae*, was isolated from soil (Daejeon, Korea). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain PB92^T was associated with the genus *Pedobacter* and was most closely related to the type strains *Pedobacter sandarakinus* DS-27^T (97.7 %), *Pedobacter roseus* CL-GP80^T (97.5 %) and *Pedobacter suwonensis* 15-52^T (97.5 %). The major cellular fatty acid components of strain PB92^T were $C_{16:1}\omega7c$ (21.4 %), iso-$C_{15:0}$ (30.8 %), iso-$C_{17:0}$ 3-OH (9.3 %) and iso-$C_{15:0}$ 2-OH (11.2 %). The G+C content of the genomic DNA from strain PB92^T was 41.4 mol%. Analysis of 16S rRNA gene sequences, as well as physiological and biochemical tests, indicated that strain PB92^T could be differentiated genotypically and phenotypically from reference species of the genus *Pedobacter*. Strain PB92^T (=KCTC 12511^T=DSM 19486^T) is proposed as the type strain of a novel species, *Pedobacter agri* sp. nov.

The genus *Pedobacter*, in the family *Sphingobacteriaceae*, was first proposed by Steyn *et al.* (1998) and includes Gram-negative, aerobic, rod-shaped bacteria. Members of the genus *Pedobacter* contain iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH, $C_{16:1}\omega7c$ and iso-$C_{17:0}$ 3-OH as major components of their cellular fatty acids (Steyn *et al.*, 1998). Since this genus was first proposed in 1998, more than 20 species, isolated from various environments including soil (Steyn *et al.*, 1998; Ten *et al.*, 2006; Yoon *et al.*, 2006, 2007), nitrifying inoculum (Vanparys *et al.*, 2005), glaciers (Margesin *et al.*, 2003, Shivaji *et al.*, 2005), fish (Steyn *et al.*, 1998) and water (Gallego *et al.*, 2006; Hwang *et al.*, 2006; Vanparys *et al.*, 2005), have been reported. The purpose of this paper was to establish the taxonomic position of a novel strain, PB92^T, by phenotypic, genetic and chemotaxonomic analyses.

Strain PB92^T was isolated from soil (Daejeon, Korea) using 10-fold diluted R2A (Difco). Cell biomass for cellular composition analysis and DNA extraction was collected from TSBA [tryptic soy broth (TSB) solidified with 20.0 g agar l^{-1} (Difco)] plates that were incubated for 2 days.

A G-spin DNA extraction kit (iNtRON Biotechnology) was used to extract chromosomal DNA. The 16S rRNA gene was PCR-amplified from chromosomal DNA using PCR Pre-Mix (Solgent) and two universal primers for bacteria (Baker *et al.*, 2003). A BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) was used according to the manufacturer's instructions to sequence PCR products purified with a PCR purification kit (Cosmo Genetech). The automated PRISM 3730XL DNA Analyzer system (Applied Biosystems) was used to analyse the resulting reaction mixtures. Full-length 16S rRNA gene sequences were assembled using SEQMAN software (DNASTAR). Pairwise 16S rRNA gene sequence similarity with that of phylogenetic neighbours was determined using the EzTaxon server (http://www.eztaxon.org/; Chun *et al.*, 2007) and sequences from PB92^T and related taxa (NCBI database) were aligned using the multiple sequence alignment program CLUSTAL_X 1.8 (Thompson *et al.*, 1997). Phylogenetic relationships between representative *Pedobacter* species were determined using the MEGA3 software program (Kumar *et al.*, 2004). Distance matrices were determined (Kimura, 1980) and

Abbreviation: PNPG, *p*-nitrophenyl β-D-galactopyranoside.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PB92^T is EF660751.

used to elaborate dendrograms by the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis was performed using a consensus tree based on 1000 randomly generated trees in order to evaluate stability. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain PB92^T is associated with the genus *Pedobacter* (Fig. 1). Strain PB92^T was most closely related to *Pedobacter sandarakinus* DS-27^T (97.7 %), *Pedobacter roseus* CL-GP80^T (97.5 %) and *Pedobacter suwonensis* 15-52^T (97.5 %). Other species of the genus *Pedobacter* had 16S rRNA gene sequence similarities of less than 95.6 % with strain PB92^T. It has been shown that two strain pairs with 16S rRNA gene sequence similarity values of less than 98.7 % have DNA–DNA reassociation values of less than 70 % (Stackebrandt & Ebers, 2006). On this basis, strain PB92^T can be considered to represent a distinct genospecies. The G+C content of strain PB92^T was determined using HPLC as described by Mesbah & Whitman (1989) with *Escherichia coli* B (Sigma-Aldrich) as the calibration reference. The genomic DNA G+C content of strain PB92^T was 41.4 mol%, which falls within the range for the genus *Pedobacter* (36.9–44.2 mol%). For quantitative analysis of the cellular fatty acid content, cells were harvested and cellular fatty acids were subjected to saponification, methylation and extraction, as described by the Sherlock Microbial Identification system (MIDI). Fatty acids were analysed by GC (Hewlett Packard 6890) and identified using the Microbial Identification software package (Sasser, 1990). The major components of the cellular fatty acid profile were $C_{16:1}\omega7c$ (21.4 %), iso-$C_{15:0}$ (30.8 %), iso-$C_{17:0}$ 3-OH (9.3 %) and iso-$C_{15:0}$ 2-OH (11.2 %). This composition profile, with $C_{16:1}\omega7c$, iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and iso-$C_{15:0}$ 2-OH as major fatty acids, is typical of members of the genus *Pedobacter* (Steyn *et al.*, 1998). In addition to the 16S rRNA gene sequence similarity and G+C content of genomic DNA of strain PB92^T, the major fatty acid components confirm that the novel strain belongs to the genus *Pedobacter*.

Gram staining was performed by the non-staining method (Buck, 1982) and cell morphology was examined by light microscopy (Nikon). Growth at different NaCl concentrations (0.5–10 %, w/v) and pH values (4.0–13.0 at intervals of 0.5 pH units) was measured using TSB. Growth at various temperatures (5, 7, 10, 15, 25, 30, 35 and 37 °C) was measured using TSBA. Catalase activity was determined by observation of bubble formation in a 3 % hydrogen peroxide solution. Enzyme activities, acid production from carbohydrates and substrate utilization from sole carbon sources were determined using API

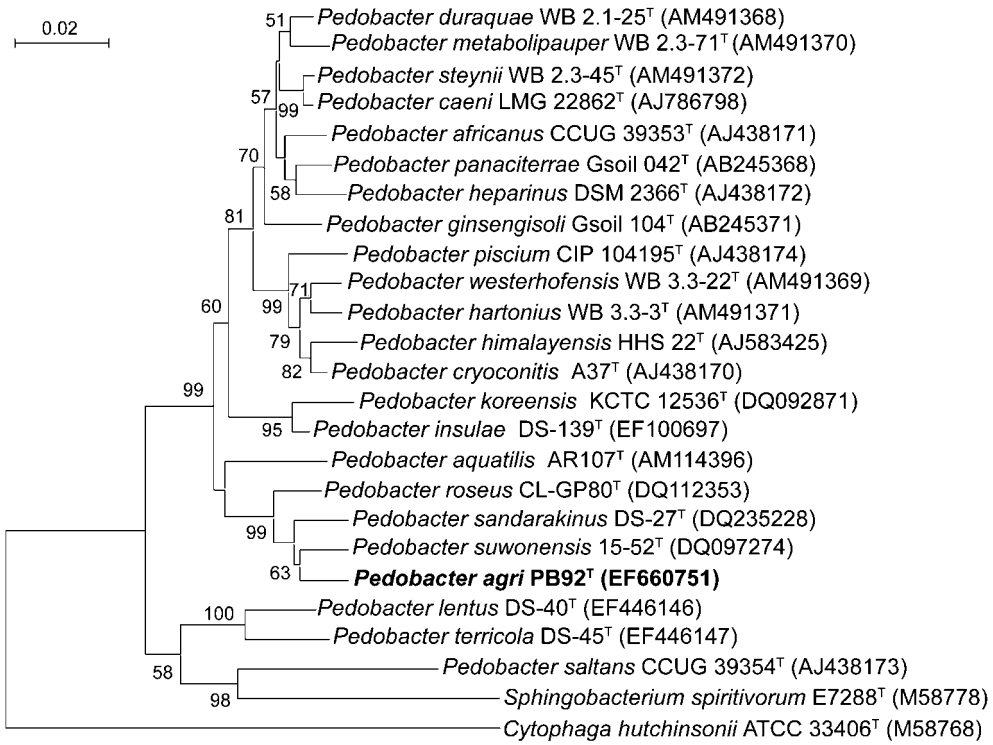

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences. The position of strain PB92^T is shown with respect to other species of the genus *Pedobacter*. The tree was generated using the neighbour-joining method. Numbers at the nodes indicate bootstrap values, expressed as percentages of 1000 replications; only values >50 % are shown. Bar, 0.02 accumulated changes per nucleotide.

20NE, API 50CH and API ZYM test strips (bioMérieux). Strain PB92^T was Gram-negative and catalase- and oxidase-negative. It did not grow in NaCl concentrations greater than 2.5 % (w/v). It was unable to reduce nitrate to nitrite or nitrogen. Strain PB92^T was positive for hydrolysis of aesculin and *p*-nitrophenyl β-D-galactopyranoside (PNPG), but negative for indole production and gelatin hydrolysis. A detailed species description is presented below. A comparison between the characteristics of PB92^T and closely related strains is given in Table 1.

Results from 16S rRNA gene sequence analysis and chemotaxonomic and phenotypic tests indicate genotypic and phenotypic differences between strain PB92^T and other species of the genus *Pedobacter*. Thus, on the basis of phenotypic, genetic and chemotaxonomic comparisons with previously described taxa, strain PB92^T represents the type strain of a novel species of the genus *Pedobacter*, for which the name *Pedobacter agri* sp. nov. is proposed.

Table 1. Differential characteristics of *Pedobacter agri* sp. nov. and closely related species

Species: 1, *Pedobacter agri* sp. nov. PB92^T; 2, *P. sandarakinus* DS-27^T (Yoon *et al.*, 2006); 3, *P. roseus* CL-GP80^T (Hwang *et al.*, 2006); 4, *P. suwonensis* 15-52^T (Kwon *et al.*, 2007); 5, *P. aquatilis* AR107^T (Gallego *et al.*, 2006). +, Positive; −, negative; W, weak reaction; NR, not reported.

Characteristic	1	2	3	4	5
Temperature range for growth (°C)	10–30	4–33	5–33	1–37	4–30
Enzyme activities:					
Leucine arylamidase	−	NR	+	+	+
Valine arylamidase	−	−	+	+	+
Cystine arylamidase	−	W	−	NR	+
Trypsin	−	−	+	+	+
α-Chymotrypsin	−	−	+	NR	−
α-Galactosidase	−	−	+	NR	−
α-Mannosidase	−	−	−	NR	+
α-Fucosidase	−	−	−	+	−
Assimilation of carbon sources:					
Glycerol	−	−	+	−	−
L-Arabinose	+	−	+	+	+
D-Ribose	−	−	+	−	−
D-Xylose	+	−	NR	+	−
L-Xylose	−	−	+	−	−
D-Adonitol	+	−	NR	−	−
D-Fructose	−	−	+	W	+
L-Rhamnose	+	−	+	+	+
Inositol	−	NR	+	NR	−
D-Mannitol	−	−	+	−	−
D-Sorbitol	−	−	+	−	−
Arbutin	+	−	NR	+	+
Inulin	−	−	+	−	−
Raffinose	W	−	+	+	+
Glycogen	W	+	+	+	−
Turanose	+	−	NR	+	+
Gluconate	−	NR	+	NR	−
5-Ketogluconate	W	+	NR	NR	−
DNA G+C content (mol%)	41.4	39.7	41.3	44.2	38.0

Description of *Pedobacter agri* sp. nov.

Pedobacter agri (a′gri. L. gen. n. *agri* of a field).

Cells are Gram-negative and rod-shaped (0.7 × 3.0 μm). Forms yellow, round colonies with a diameter of 1.0–2.0 mm after incubation for 2 days on TSBA. Grows at 10–30 °C and pH 6.0–8.0; optimal growth occurs at 25 °C and pH 7.0. No growth occurs at 7 or 35 °C or at NaCl concentrations greater than 2.5 % (w/v). Catalase- and oxidase-negative. Cannot reduce nitrate to nitrite or nitrogen, does not produce indole and does not ferment glucose. Negative for arginine dihydrolase and urease. Hydrolyses aesculin and PNPG, but not gelatin. Utilizes L-arabinose, D-xylose, D-adonitol, D-galactose, D-glucose, D-mannose, L-rhamnose, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, D-lactose, melibiose, sucrose, trehalose, melezitose, raffinose, starch, glycogen, gentiobiose, turanose and 5-ketogluconate as sole carbon sources, but not glycerol, erythritol, D-arabinose, D-ribose, L-xylose, methyl β-D-xyloside, D-fructose, L-sorbose, dulcitol, inositol, D-mannitol, D-sorbitol, inulin, xylitol, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate or 2-ketogluconate. Assays using the API ZYM system are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase and naphthol-AS-BI-phosphohydrolase. In contrast, lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are not observed. The predominant fatty acids are iso-$C_{15:0}$ (30.8 %), $C_{16:1}\omega7c$ (21.4 %), iso-$C_{15:0}$ 2-OH (11.2 %) and iso-$C_{17:0}$ 3-OH (9.3 %). $C_{14:0}$ (1.6 %), $C_{16:0}$ (2.6 %), $C_{16:0}$ 3-OH (2.7 %), iso-$C_{15:0}$ 3-OH (2.0 %), iso-$C_{16:0}$ 3-OH (0.9 %), anteiso-$C_{15:0}$ (1.3 %) and $C_{16:1}\omega5c$ (3.3 %) are also present.

The type strain is PB92^T (=KCTC 12511^T=DSM 19486^T), isolated from soil (Daejeon, Korea). The G+C content of the genomic DNA from the type strain is 41.4 mol%.

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